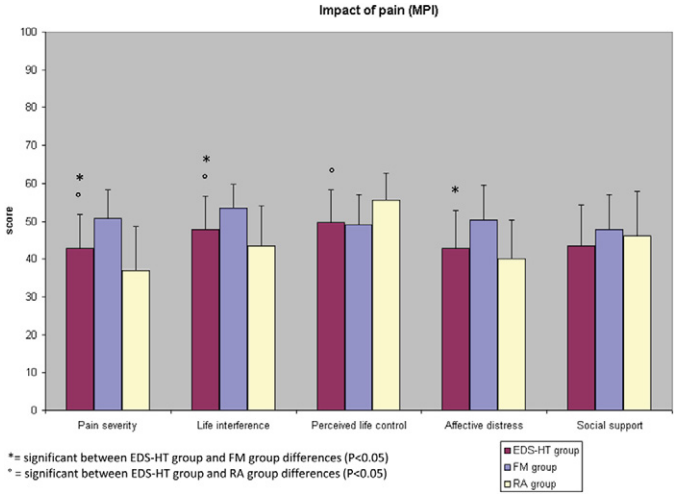

Figure 1

and pain-related suffering, significantly more interference of pain with daily activities, and significantly lower control over pain and life events compared to the RA group. However, in comparison with the FM group, the EDS-HT group showed significantly lower levels of pain severity and life interference, and was less bad-tempered, over-sensitive and anxious ('affective distress'). Social support of significant others due to pain was similar between the 3 groups.

The results in Figure 2 showed clinically relevant health-related dysfunction in all groups. Especially, a significantly poorer physical, psychosocial and overall health function was found in the EDS-HT group compared with the RA group. In contrast, in comparison with the FM group, the EDS group reported a similar physical and overall health status, but a better psychosocial health.

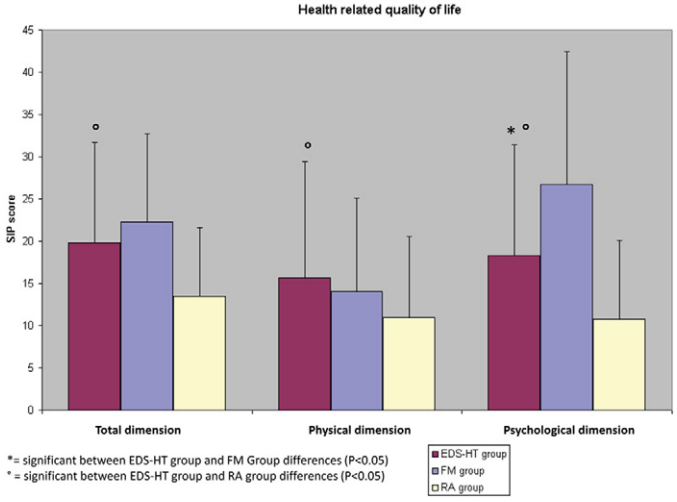

Figure 2

Conclusions: This study demonstrates that patients with EDS-HT experience a broad psychosocial impact of chronic pain on daily life, similar or even worse compared to patients with FM or RA. At the same time, this study shows that EDS-HT has a considerable impact on physical and psychosocial health in most areas of HRQOL. Our findings emphasize that EDS-HT, like other chronic widespread musculoskeletal pain disorders, is associated with a consistent burden of disease. Therefore, treatment of pain should be a prominent aspect of symptomatic management of EDS-HT.

Proteomics & Metabolomics

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PROTEOMIC PROFILING OF CARTILAGE EXTRACELLULAR MATRIX MATURATION

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Purpose: In recent years there has been a rapid expansion in the use of proteomics to discover biomarkers and new molecular mechanisms involved in joint disease. In addition, proteomic discovery of novel cartilage components and interactions that have evaded detection by more targeted biochemical approaches will facilitate analysis of biomaterials developed for cartilage repair. To gain a more complete understanding of cartilage extracellular matrix (ECM) maturation, we analyzed changes in protein expression and solubility in a novel mouse neo-cartilage system.
Methods: Primary mouse chondrocytes were maintained in scaffold-free, high density cultures for up to 6 weeks and transmission electron microscopy was used to verify production of a cartilaginous ECM. Sequential extracts of juvenile cartilage (P3) and 3-week neo-cartilage, prepared using 1M NaCl followed by 4M GuHCl, were initially analysed by SDS-PAGE. Label-free quantitative mass spectrometry (LTQ-Orbitrap), statistical and bioinformatic analysis was then used to filter out three significant protein groups: proteins enriched according to extraction condition ("extraction profiling"), proteins differentially abundant between juvenile cartilage and neo-cartilage, and proteins with different solubility properties between the two tissues. Key proteins were further investigated in neo-cartilage and 3-week mouse femoral head cartilage by immunohistochemistry.
Results: Ultrastructural analysis revealed clearly defined pericellular and territorial matrix zones, dense proteoglycan/collagen networks and intricate chondrocyte-matrix contacts. SDS-PAGE indicated that in juvenile cartilage, more proteins were readily soluble, whereas in the neo-cartilage more proteins were extracted under denaturing conditions. LTQ MS/MS identified a total of 819 proteins at high confidence (2 or more unique peptides), of which 620 and 706 proteins were detected in juvenile cartilage and neo-cartilage, respectively. Proteins significantly enriched in neo-cartilage ($n=78$, $p<0.05$ using Student's t-test) included proteins previously not reported or with unknown function in cartilage (EDIL3, integrin-binding protein DEL1; CCD80, coiled-coil domain-containing protein 80; EMIL1, elastin microfibril interface-located protein 1 and PEDF, pigment epithelium-derived factor). The cohort of proteins with the greatest differential in extractability between the two sample types included many pericellular and extracellular matrix components, including collagen VI, nidogen-2, perlecan, matrilin-3 and COMP. One of the guanidine extract specific proteins in the mouse neo-cartilage was the serine protease inhibitor, protease nexin-1. We confirmed PN-1 as a novel component of developing articular cartilage in vitro and in vivo by immunohistochemistry.
Conclusions: The cartilage "extraction profiles" are, to our knowledge, the most detailed solubility-based comparative analysis of a tissue proteome. The partitioning of readily soluble proteins from more tightly-integrated components applied to cellular components of large protein complexes (eg tubulins, ribosomal and proteasomal subunits) as well as ECM proteins and proteoglycans. This fractionation approach therefore facilitates deeper mining of the proteome while maintaining important biochemical information related to the proteins identified. Using these differences in protein solubility we generated a comprehensive profile of mouse neo-cartilage and identified novel components involved in maturation of the cartilage ECM.

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STUDY OF THE EFFECT OF CHONDROITIN SULFATE ON CARTILAGE EXTRACELLULAR MATRIX METABOLISM BY CHONDROCYTE SECRETOME ANALYSIS: USEFULNESS OF A QUANTITATIVE PROTEOMIC APPROACH

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Purpose: To study the effect of chondroitin sulfate (CS) on the profile of

chondrocyte secreted proteins using the stable isotope labelling by amino acids in cell culture (SILAC) technique.
Methods: Cartilage obtained from patients undergoing joint replacement, or from patients with no history of joint disease was provided by the Tissue Bank and the Autopsy Service at CHU A Coruña. The study was approved by the local Ethics Committee. Chondrocytes released from cartilage by enzymatic digestion were recovered and plated at low density in basic SILAC medium (Silantes) supplemented with antibiotics and 10% FBS dialyzed. In the case of light media, standard L-lysine (146 mg/L) and L-arginine (28 mg/L) were used, while in the heavy media isotope-labelled L-lysine ($^{13}C_6$), and isotope-labelled L-arginine ($^{13}C_6$,$^{15}N_4$) were used. When complete incorporation of the heavy isotope was achieved in the cells (2-3 weeks), normal (N) chondrocytes were treated with CS 200μg/mL and then stimulated with IL-1β 5ng/mL, while osteoarthritic (OA) chondrocytes were treated with CS 200μg/mL alone. 48 hours later, conditioned media were collected and their proteins were concentrated and quantified. Heavy and light samples were mixed 1:1, and 4 μg of each mixed sample were in-solution reduced, alkylated and digested with trypsin. Separation and analysis of the resulting tryptic peptide mixtures was performed by nanoscale reversed-phase-LC-MS/MS. The identification and quantification of proteins was carried out with Protein Pilot software, which detects the heavy/light peak pairs and calculates the heavy/light ratios based on the peak areas. Identifications with a probability score higher than 95% and quantifications with a p value $\leq$0.05 were included in the results list.
Results: Database search (UniprotKB/Swissprot) allowed us the identification of 39 different proteins in the OA chondrocyte secretome and 70 in N chondrocyte secretome. Interestingly, in both cases the most abundant protein was cartilage glycoprotein 39, which has been previously related with OA pathogenesis. For biological and functional analysis we considered only those proteins detected in all replicates with a heavy/light ratio $\geq$1.2 or $\leq$0.8. In OA chondrocytes, chondroitin sulfate mainly improves the anabolic/catabolic balance of the extracellular cartilage matrix, by increasing the level of structural proteins like collagens, decorin, lumican, vimentin and fibronectin. In N chondrocytes stimulated with IL1β, CS appears to act primarily as an anti-inflammatory drug. We show in this work how CS reduces inflammation by two mechanisms: directly, by decreasing the presence of potent inflammatory mediators like IL6 (ratio=0.6), and also indirectly, by increasing proteins such as tumor necrosis factor α-induced protein (TSG6, ratio=3). TSG6 plays a crucial role in extracellular matrix formation, inflammatory cell migration and cell proliferation. It's a key component of a negative feedback loop operating through the protease network which reduces matrix degradation during OA process. The mechanism driven by TSG6 leads to a decrease in proMMPs activation, which might protect cartilage from extensive degradation even in the presence of acute inflammation (represented in our case by a high level of IL1β).
Conclusions: We have carried out the first pharmacoproteomic study using a quantitative proteomics approach (SILAC), based on the metabolic labelling of the cells, to study the effect of CS on chondrocyte secretome. Our findings provide novel information about the mechanisms that may exert the *in vivo* beneficial effects of CS on the OA disease process. This work also illustrates that chondrocyte secreted proteins are an attractive sub-proteome for the discovery of new targets of CS in OA therapy.

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QUANTITATIVE VALIDATION OF PROTEINS IDENTIFIED IN THE CARTILAGE SECRETOME IN AN EXPLANT MODEL OF EARLY OSTEOARTHRITIS

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Purpose: Previous in-house proteomic work identified several secreted proteins from equine cartilage explants in response to recombinant equine interleukin-1 beta (IL-1β) with or without the non-steroidal anti-inflammatory drug, carprofen. The aim of this study was to validate the presence of six of these proteins by western blotting. We also used quantitative densitometry to determine the effects of IL-1β, with or without carprofen treatment, on levels of these proteins in the cartilage secretome compared to control explants.
Methods: Cartilage explants were obtained from weight-bearing regions of metacarpophalangeal joints of horses euthanized for purposes other than for research. Explants were either incubated alone (control: C), with IL-1β (10ng/ml), or in combination with IL-1β and carprofen (IL-1β+ CA, 10ng/ml and 100μg/ml respectively) at 37°C for 5 days. Culture medium supernatants were collected and each sample divided into two aliquots. One aliquot underwent tryptic digestion and high-throughput proteomic analysis by ESI (Electrospray Ionisation) mass spectrometry using a Bruker HCT PTM discovery ion trap instrument. Comparative proteomic analysis of the supernatants identified a number of potentially relevant proteins. The remaining corresponding aliquots were resolved on 1-D gels and either silver stained to compare their electrophoretic profiles or used for western blotting to validate protein expression. Six of the most commonly identified proteins were selected for quantitative validation by western blotting; cartilage oligomeric matrix protein (COMP), thrombospondin-1 (TSP-1), clusterin (mature and precursor forms), cartilage intermediate layer protein-1 (CILP-1) and the matrix metalloproteinases MMP-1 and MMP-3.
Results: SDS-PAGE and silver staining revealed qualitative differences between the electrophoretic profiles of samples exposed to the different treatments. Western blotting confirmed the presence of COMP, TSP-1, clusterin, clusterin precursor, CILP-1, MMP-1 and MMP-3 in explant supernatants. Quantitative densitometry indicated that TSP-1, MMP-1 and MMP-3 levels were increased in IL-1β and IL-1β+CA samples compared to controls. Carprofen reduced MMP-1 and MMP-3 levels in IL-1β+CA compared to IL-1β treatment alone. CILP-1 and clusterin levels remained unchanged in all treatments, although the clusterin precursor was decreased in IL-1β samples.

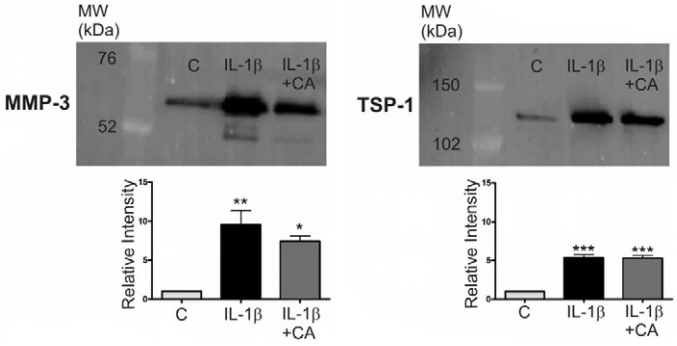

Figure 1

Conclusions: The authors' previous proteomic work has identified several relevant extracellular matrix proteins in explant supernatants stimulated with IL-1β. This study confirmed the presence of six of these proteins by quantitative western blotting and densitometry. Many of the identified proteins have well-known matrix functions including participation in cell-matrix and matrix-matrix interactions (i.e. TSP-1, COMP, CILP), matrix turnover (MMP-1, MMP-3) and extracellular molecular chaperone activity (clusterin). The validation described in this study suggests that this high-throughput proteomic system provides a useful tool to identify candidate proteins from the cartilage secretome for further quantitative analysis using western blotting.
Acknowledgements: This work was supported by the BBSRC (BBS/S/M/2006/13141) and the WALTHAM Centre for Pet Nutrition.

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COMPREHENSIVE ANALYSIS OF THE INTERLEUKIN-1-BETA-MEDIATED MODULATION OF CHONDROCYTE INTRACELLULAR AND EXTRACELLULAR PROTEOMES BY METABOLIC LABELLING

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Purpose: The aim of this study is to standardize the stable isotope labelling by amino acids in human chondrocytes cell culture (SILAC) technique, and to apply this novel strategy for the study of osteoarthritis (OA) pathophysiology. To attain this objective, we have used an *in vitro* model of inflammation based on the stimulation of human articular chondrocytes with the cytokine Interleukin-1β (IL1β), a key OA mediator.
Methods: Cartilage obtained from patients with no history of joint disease